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PP2A-dependent transactivation of the cyclin A promoter by SV40 ST is mediated by a cell cycle-regulated E2F site

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Abstract

The Simian Virus 40 (SV40) small-t antigen (ST) plays an important role in driving cell proliferation, enhancing transformation by the large-T (LT) antigen. Potential targets of ST are the cyclin kinase inhibitor p27 and the cyclin A gene itself. Transactivation of the cyclin A promoter by ST depends on the interaction of ST with protein phosphatase 2A (PP2A) and occurs through a cell cycle-regulated E2F site near the transcription start site of the promoter. A third SV40 early protein, 17KT, also transactivates the cyclin A promoter but, in this case, transactivation depends on the dnaJ domain of the protein.

Keywords: PP2A-dependent transactivation; SV40 ST; E2F site

Introduction

The contributions of the Simian Virus 40 (SV40) small-t (ST) antigen to cellular transformation were only fully appreciated as studies of SV40 moved into human cell systems. Both LT and ST are required to transform human fibroblasts (Chang et al., 1985; DeRonde et al., 1989; Ozer et al., 1996; Porras et al., 1996), but the domains of ST critical for this function have been explored only recently (Porras et al., 1996). The requirement for ST in transformation is more stringent in human cells than in rodent cells, and ST is required to induce both focus formation and anchorage-independent growth. This joint requirement is mirrored by the stimulation of cell cycle progression by SV40 (Rundell et al., 1998), where LT and ST are both needed to overcome cell cycle inhibition. Density-arrested fibroblasts only escape contact inhibition when LT and ST cooperate to lower the cyclin-dependent kinase inhibitors p21 and p27, respectively (Porras et al., 1999). Studies on the role of ST in human cell transformation have now been extended to other cell types as well (Hahn et al., 2002; Yu et al., 2001).

The effects of ST on host cells are primarily mediated by its regulation of protein phosphatase 2A (PP2A) (Kamibayashi et al., 1992; Yang et al., 1991). ST sequences between residues 97 and 103 are involved in binding the PP2A-A subunit, and mutations like C103S have been used extensively to study this ST function (Mungre et al., 1994; Porras et al., 1996; Yu et al., 2001). Indeed, the ability of ST to interact with PP2A has been found to be critical in all ST-dependent transformation systems tested to date (Hahn et al., 2002; Mungre et al., 1994; Porras et al., 1996; Yu et al., 2001). Generally, ST binding reduces enzymatic activity leading to an increase in the levels of active, phosphorylated forms of key cellular proteins, e.g., MAPK, the Na/H-antiporter, and AKT (Howe et al., 1998; Sontag et al., 1993; Yuan et al., 2002). MAPK and AKT have several important downstream targets. For example, ST can drive transcription of AP1-regulated promoter constructs (Frost et al., 1994) in a MAPK-dependent fashion, as illustrated in studies of the cyclin D1 promoter (Watanabe et al., 1996).

ST also contains a dnaJ domain within amino-terminal sequences that are shared with LT (Kelley and Georgopoulos,

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1997). In LT, the dnaJ domain participates in the targeting of the Rb family members, p130 and p107, for degradation (Stubdal et al., 1996, 1997), resulting in the relief of repression by Rb family members and enhanced cell proliferation. Recently, it was recognized that SV40 encodes a 17KT protein (Zerrahn et al., 1993) and that this protein also targets p130 for degradation and promotes cell proliferation (Boyapati et al., 2003). No clear role has been assigned to the dnaJ domain in ST although an earlier study suggested that this domain might function in the transactivation of the cyclin A promoter (Porras et al., 1996). In this report, we reevaluate this study based on our current understanding that the 17KT protein is expressed from some plasmids thought to encode ST alone. We now report that both ST and the 17KT protein can transactivate this promoter, ST in a PP2A-dependent fashion and 17KT through the dnaJ domain. In addition, the site in the cyclin A promoter that responds to ST was mapped to an E2F site previously identified as a cell cycle-regulated element (Schulze et al., 1995).

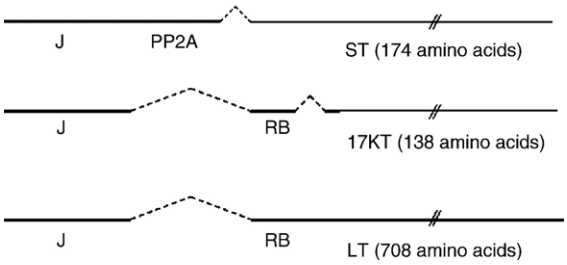

Fig. 1. Diagram of SV40 early region products. In this diagram of the SV40 early region proteins, bold-faced lines represent the coding regions of each protein, ST, 17KT, and LT (total amino acid content for each protein is indicated, and lines are not drawn to scale). Dashed lines indicate the introns that are removed from the common precursor mRNA for these proteins. Non-coding regions of the transcripts are shown by thin lines. The general positions of the dnaJ domain (J), PP2A, and RB binding sites are indicated below the bold-faced lines.

Results and discussion

Early studies of human fibroblasts infected with recombinant adenoviruses demonstrated that ST alone can increase levels of cyclin A protein in cells, even when cell cycle progression continues to be restricted by p21 (Porras et al., 1999). This increase was reflected in the levels of cyclin A mRNA. Moreover, the ST-expressing plasmid pw2t increased transcription from reporter constructs that contained the cyclin A promoter. Surprisingly, initial studies using pw2t C103S suggested that PP2A interaction was not involved in ST-induced transactivation of the cyclin A promoter. Rather, the dnaJ domain found in amino-terminal sequences shared by ST and LT appeared to be responsible because a ST-expressing plasmid harboring a mutation in this domain, pw2t 43/45 (P43L/K45N), showed reduced transactivation (Porras et al., 1996). It was hard to reconcile these findings with the fact that the PP2A-binding domain, but not the dnaJ domain, of ST was required to drive cell cycle progression in the presence of LT when both SV40 proteins were individually expressed from recombinant adenoviruses (Rundell et al., 1998).

Some of the inconsistencies we found in the pattern of transactivation now appear to reflect the expression of a third SV40 early region product, the 17KT protein (Zerrahn et al., 1993). As shown in Fig. 1, 17KT is a small protein which resembles LT in having dnaJ and Rb binding domains, but a second splice in the message alters the reading frame of the protein thus deleting most of the carboxyterminal sequences found in LT. The need to examine a role for 17KT in cyclin A transactivation was first indicated by experiments comparing two plasmids, pw2t and pw2tDL888 (pw2tDL), in which the splice donor for ST mRNA is deleted, totally eliminating ST expression. Before the discovery of the 17KT protein, we believed that the pw2tDL plasmid did not express any SV40 proteins. However, as shown in Fig. 2A, both plasmids activated transcription of the luciferase gene from the 8.1 Kb cyclin A promoter. Furthermore, transactivation by pw2tDL was nearly eliminated by mutation of the LXCXE motif to which Rb family members bind (Fig. 2B, pw2tDLRb-). These findings, and parallel studies of the behavior of these constructs in transformation assays, led us to reinvestigate SV40 protein expression by pw2tDL (Boyapati et al., 2003). No SV40 proteins were found in pw2tDL-transfected cells by 35S-labeling or Western blotting. However, ^{32}P-labeled cells were found to express low levels of a 17KT phosphoprotein, as described by Zerrahn et al. (ST itself is not a phosphorylated protein).

The transactivation of the cyclin A promoter by pw2tDL suggested that the 17KT protein might be responsible. This possibility seemed likely because 17KT resembles LT in having dnaJ and pRb binding domains, and LT is a good transactivator of the cyclin A promoter as well (Beachy et al., 2002). In addition, we showed that 17KT could alter stability of the Rb family member, p130, and drive cell cycle progression in the absence of other viral proteins (Boyapati et al., 2003). Thus, 17KT resembled the better studied LT protein in these activities (Stubdal et al., 1996, 1997). To study ST and 17KT proteins individually, we developed cDNA derivatives of each protein (Boyapati et al., 2003). These constructs showed that either ST or 17KT could activate transcription from the cyclin A promoter (Fig. 3A). Furthermore, transactivation by the 17KT was dependent on the dnaJ region of this protein and was significantly reduced by the 43/45 mutation. It was also reduced by mutation of the Rb binding motif (data not shown). In these experiments, we used a 17KT cDNA derivative that also contains an intron upstream of the ATG translational start site. This construct was derived previously because we were unable to detect 17KT protein from a cDNA construct in the absence of an intron. Although not shown here, we confirmed that the 43/45 dnaJ mutation eliminated transactivation by pw2tDL as well, consistent with the idea that the 17KT

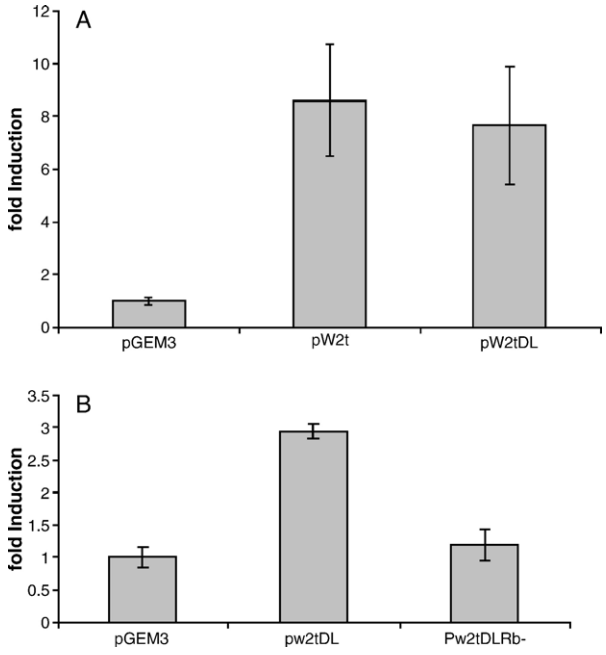

Fig. 2. Transactivation of the cyclin A promoter by pw2t constructs. (A) TC7 cells were cotransfected with PALUC and pw2t, which is known to express both ST and 17KT, or pw2tDL, in which the ST splice donor is absent but 17KT expression is unaffected. (B) TC7 cells were cotransfected with PALUC and either pw2tDL or pw2tDLRb-, a mutation in the LXCXE Rb-binding motif, to show the dependence of transactivation on this motif when ST is not expressed. For both A and B, cell lysates were extracted 48 h post-transfection. Results are expressed relative to the pGEM3 control plasmid.

protein expressed by this plasmid drives the cyclin A promoter.

Transactivation of the cyclin A promoter by both 17KT and ST required that we re-interpret earlier mutational studies. In particular, it was not possible to determine whether the PP2A-binding region of ST played any role because plasmids that carried mutations in this region continued to express WT 17KT. Therefore, cDNA constructs which express only ST were used to explore mechanisms behind the transactivation of the cyclin A promoter by ST, the focus of the rest of this study. As shown in Fig. 3B, the transactivation of the cyclin A promoter by ST alone was eliminated by C103S mutation, clearly demonstrating the requirement for the interaction of ST with PP2A. In contrast, the dnaJ domain mutation had only a slight effect on transactivation of the cyclin A promoter by ST. Thus, earlier reports describing a role of the dnaJ domain in cyclin A transactivation reflected the activity of the 17KT protein and not that of ST itself.

The cell cycle regulation of the cyclin A promoter has been previously studied in detail (Henglein et al., 1994). This study defined the cell cycle-regulated regions in the 8.1 kb promoter by testing a series of truncated promoter/luciferase constructs for their responsiveness at the onset of S-phase. The promoter proximal sections of this promoter are diagrammed in Fig. 4A. Initial studies tied cell cycle regulation of the cyclin A promoter to a 180-bp region consisting of 117 nucleotides upstream from the transcription start site and 30 nucleotides downstream (Henglein et al., 1994). Within this region were an ATF site (−70) and a variant E2F site (−37). A subsequent study established that the variant E2F site binds to nuclear proteins extracted from cells at G1/S and that cell cycle regulation is lost when this E2F site is mutated from TCGCG to AGCTT (Schulze et al., 1995). This site will be referred to as the cell cycle-regulated E2F site or E2F* for the rest of this discussion.

To ascertain which regions of the cyclin A promoter respond to ST, each of the truncated cyclin A promoter/luciferase constructs was screened for transactivation in response to pw2t cDNA. Although actual data with these original constructs are not shown here, the smallest ST-responsive promoter fragment (−215 to +245) was one that contained three Sp1 sites, an ATF site and E2F*, along with minor transcription factor binding sites that fall between +11 (transcription start site) and +245. Mutation of the three Sp1 sites in this fragment did not eliminate ST-induced transactivation (data not shown).

Once these initial experiments were conducted using the available PALUC truncations, smaller regions of the promoter were cloned by PCR to better define the ST-responsive elements. The shortest derivative that responded

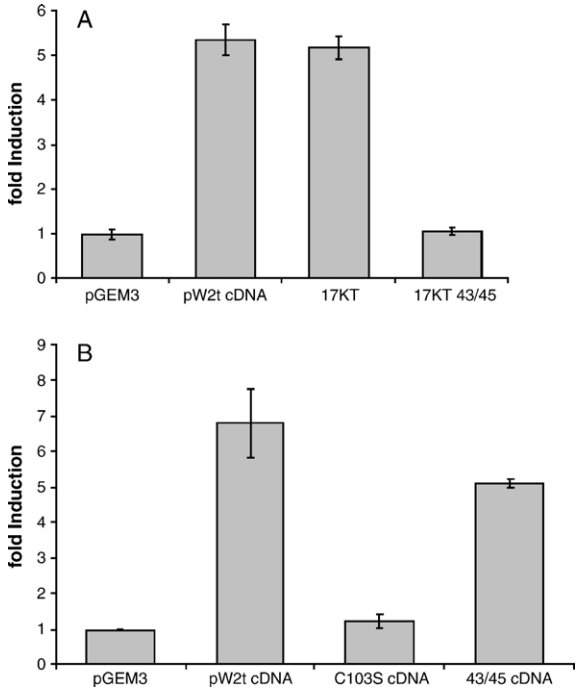

Fig. 3. Transactivation of the cyclin A promoter by ST or 17KT individually. (A) TC7 cells were cotransfected with PALUC and cDNAs for either ST (pw2t cDNA) or the 17KT protein. The dependence of 17KT transactivation on the dnaJ domain was tested using 17KT 43/45. (B) TC7 cells were cotransfected with PALUC and plasmids that encode cDNAs for WT ST, the PP2A binding mutant C103S, or the dnaJ domain mutant P43L/K45N. For both A and B, cell lysates were collected 48 h post-transfection. Results are expressed relative to the pGEM3 control.

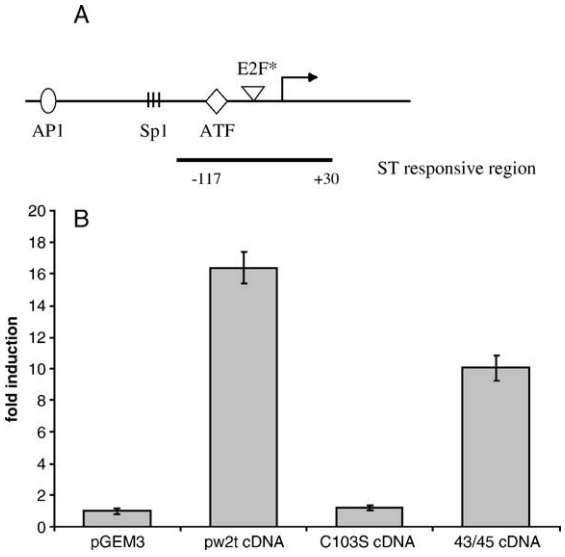

Fig. 4. ST transactivation of a minimal cyclin A promoter. (A) The diagram shows major transcription factor binding sites in the cyclin A promoter, from an AP1 site at about −300 (relative to the transcription start site) to the start of translation at +245. The solid line marks the limits of the minimal small-t-responsive promoter construct used in B. The arrow above the line indicates the start of transcription. (B) Luciferase activity was measured from a construct that contains cyclin A promoter sequences −117 to +30 as diagrammed in A. TC7 cells were cotransfected with this reporter and plasmids that expressed WT ST, C103S, or P43L/K45N. Cell lysates were collected 48 h post-transfection. Results are expressed relative to the pGEM3 control which was set at 1. The background levels differ because the mutation leads to derepression of the promoter.

to ST included bases −117 to +30, shown in Fig. 4A. This construct contains only an ATF site and the cell cycle-regulated E2F site described in earlier studies. As shown in Fig. 4B, this minimal promoter was readily transactivated by the pw2t cDNA plasmid, and transactivation was strongly dependent on the interaction of ST with PP2A. Although the mutated dnaJ domain of ST slightly reduced transactivation, the reduction was modest and far less striking than that of the PP2A mutation.

Results with this minimal promoter have several implications. First, transactivation of the cyclin A promoter by ST does not depend on the Sp1 sites. Others have reported that ST has the ability to stimulate transcription of other promoters via Sp1 (Garcia et al., 2000). Apparently, the Sp1 sites are not the major ST-responsive sequences in the cyclin A promoter. Second, any minor transcription factor binding sites found between +30 and +245 of the promoter are not required. Finally, the role of the ATF sequence at −77 is of interest. Mutations of this site reduced basal transcription so dramatically that it was difficult to monitor ST responsiveness, although experiments reproducibly showed greater activity from ATF mutant promoters when ST was present (data not shown). Taken together, approaches using truncated promoters suggested that the cell cycle-regulated E2F site was the primary target for regulation by ST.

In order to fully explore the responsiveness of the E2F* site to ST, the point mutations ablating this site were introduced into a cyclin A promoter/luciferase construct. The backbone promoter used contains over 7 kb of upstream sequences relative to the transcription start site but only 11 nt downstream. It is nearly identical to the promoter used in Figs. 2 and 3, except that it lacks a few hundred nucleotides downstream from the transcription start site. These sequences were not required for cell cycle regulation (Henglein et al., 1994) or for ST transactivation. As shown in Fig. 5, the WT promoter, E2F*wt-LUC, was fully responsive to ST and, although not shown here, this response was dependent on the PP2A binding ability of ST. In contrast, E2F*mut-LUC showed significantly less transactivation by ST. To produce this mutant, the E2F sequence at −37 was changed from tagTCGCGgga to tagAGCTTgga (Schulze et al., 1995). The nearly full-length promoter used in this experiment contains many other transcription factor binding sites (AP1, Sp1, ATF) that are potential targets for PP2A regulation, yet mutation of the single cell cycle-regulated E2F site had a pronounced effect. The use of this 7KB promoter, rather than a truncated derivative, highlights the importance of the cell cycle-regulated E2F site in ST responsiveness.

In summary, it appears that the sequences in the cyclin A promoter that are responsible for cell cycle regulation and those targeted by ST are identical. It is intriguing that the variant E2F site has been found to be regulated, at least in part, by the cki p27 which regulates the presence of cyclin E-cdk2 complexes formed at this site in G1 (Zerfass-Thome et al., 1997). This raises the possibility that ST, which has been shown to decrease levels of p27 in cells (Porras et al., 1999), is not directly acting at the promoter but that it is able to increase transcription from this promoter by inhibiting p27.

Materials and methods

Cell culture

All transactivations were performed using CV1 monkey kidney cells, TC7 clone, kindly provided by Dr. M. Judith

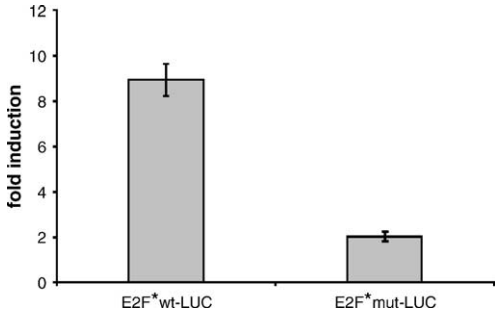

Fig. 5. Role of the cell cycle-regulated E2F site in ST transactivation of the cyclin A promoter. TC7 cells were transfected with a reporter vector which contains the 7 kb cyclin A promoter region up to +11, or the variant in which the cell cycle-regulated E2F site was mutated. Results are expressed relative to the pGEM3 control which was set to 1.

Tevethia. Cells were grown in Dulbecco's modified Eagle's medium (DME) containing 5% fetal bovine serum.

Plasmids

Derivatives of the p2wt plasmids (Chang et al., 1985) were described previously (Boyapati et al., 2003; Porras et al., 1996). Luciferase reporters containing various derivatives of the cyclin A promoter have also been described (Henglein et al., 1994; Schulze et al., 1995). The minimally responsive promoter described in Fig. 3 was constructed by cloning cyclin A sequences obtained by polymerase chain reaction upstream of a minimal TATA box in the luciferase-expression plasmid TK81 (Watanabe et al., 1996).

Transactivation

TC7 cells were plated 16–18 h prior to transfection using 4.5×10^5 cells per 60 mm dish then transfected as described (Cavender et al., 1999). DNA (10 μg total) was suspended in buffer then adjusted to a final concentration of 500 μg/ml DEAE-dextran. This solution was then added to twice-washed cells for 20 min at room temperature. All plasmids used were prepared by double-banding in cesium chloride-ethidium bromide gradients. The experiments described here were done using 2.5 μg luciferase reporter construct, 5 μg ST or 17KT expression plasmid, and 2.5 μg salmon-sperm DNA.

Luciferase assays

Luciferase assays were performed using the Luciferase Assay System (Promega) and luminescence was read using a Bio-TEK Synergy HT Multidetection Microplate Reader. The concentrations of protein extracts were determined using the Bio-Rad reagent, and equal amounts of each lysate were used in the assay. Quadruplicate aliquots of each extract were assayed in each experiment and used to determine statistical significance. In addition, each experiment was repeated at least three times, and the experiments shown are representative of the replicate experiments.

Acknowledgments

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References

Beachy, T.M., Cole, S.L., Cavender, J.F., Tevethia, M.J., 2002. Regions and activities of simian virus 40 T antigen that cooperate with an activated ras oncogene in transforming primary rat embryo fibroblasts. J. Virol. 76, 3145–3157.

Boyapati, A., Wilson, M., Yu, J., Rundell, K., 2003. SV40 17KT antigen complements dnaj mutations in large T antigen to restore transformation of primary human fibroblasts. Virology 315, 148–158.

Cavender, J.F., Mummert, C., Tevethia, M.J., 1999. Transactivation of a ribosomal gene by simian virus 40 large-T antigen requires at least three activities of the protein. J. Virol. 73, 214–224.

Chang, L.S., Pan, S., Pater, M.M., Di Mayorca, G., 1985. Differential requirement for SV40 early genes in immortalization and transformation of primary rat and human embryonic cells. Virology 146, 246–261.

DeRonde, A., Sol, C.J.A., van Strien, A., ter Schegget, J., van der Noordaa, J., 1989. The SV40 smallt antigen is essential for the morphological transformation of human fibroblasts. Virology 171, 260–263.

Frost, J.A., Alberts, A.S., Sontag, E., Guan, K., Mumby, M.C., Feramisco, J.R., 1994. Simian virus 40 small t antigen cooperates with mitogen-activated kinases to stimulate AP-1 activity. Mol. Cell. Biol. 14, 6244–6252.

Garcia, A., Cereghini, S., Sontag, E., 2000. Protein phosphatase 2A and phosphatidylinositol 3-kinase regulate the activity of Sp1-responsive promoters. J. Biol. Chem. 275, 9385–9389.

Hahn, W.C., Dessain, S.K., Brooks, M.W., King, J.E., Elenbaas, B., Sabatini, D.M., DeCaprio, J.A., Weinberg, R.A., 2002. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Mol. Cell. Biol. 22, 2111–2123.

Henglein, B., Chenivesse, X., Wang, J., Eick, D., Brechot, C., 1994. Structure and cell cycle-regulated transcription of the human cyclin A gene. Proc. Natl. Acad. Sci. U.S.A. 91, 5490–5494.

Howe, A.K., Gaillard, S., Bennett, J.S., Rundell, K., 1998. Cell cycle progression in monkey cells expressing simian virus 40 small t antigen from adenovirus vectors. J. Virol. 72, 9637–9644.

Kamibayashi, C., Lickteig, R.L., Estes, R., Walter, G., Mumby, M.C., 1992. Expression of the A subunit of protein phosphatase 2A and characterization of its interactions with the catalytic and regulatory subunits. J. Biol. Chem. 267, 21864–21872.

Kelley, W.L., Georgopoulos, C., 1997. The T/t common exon of simian virus 40, JC and the BK polyomavirus T antigens can functionally replace the J-domain of the *Escherichia coli* DnaJ molecular chaperone. Proc. Natl. Acad. Sci. U.S.A. 94, 3679–3684.

Mungre, S., Enderle, K., Turk, B., Porras, A., Wu, Y.-Q., Mumby, M.C., Rundell, K., 1994. Mutations which affect the inhibition of protein phosphatase 2A by simian virus 40 small-t antigen in vitro decrease viral transformation. J. Virol. 68, 1675–1681.

Ozer, H.L., Banga, S.S., Dasgupta, T., Houghton, J., Hubbard, K., Jha, K.K., Kim, S.H., Lenahan, M., Pang, Z., Pardinas, J.R., Patsalis, P.C., 1996. SV40-mediated immortalization of human fibroblasts. Exp. Gerontol. 31, 303–310.

Porras, A., Bennett, J., Howe, A., Tokos, K., Bouck, N., Henglein, B., Sathyamangalam, S., Thimmapaya, B., Rundell, K., 1996. A novel simian virus 40 early-region domain mediates transactivation of the cyclin A promoter by small-t antigen and is required for transformation in small-t antigen-dependent assays. J. Virol. 70, 6902–6908.

Porras, A., Gaillard, S., Rundell, K., 1999. The simian virus 40 small-t and large-T antigens jointly regulate cell cycle reentry in human fibroblasts. J. Virol. 73, 3102–3107.

Rundell, K., Gaillard, S., Porras, A., 1998. Small-t and large-T antigens cooperate to drive cell proliferation. Dev. Biol. Stand. 94, 289–295.

Schulze, A., Zerfass, K., Spitkovsky, D., Middendorp, S., Berges, J., Helin, K., Jansen-Durr, P., Henglein, B., 1995. Cell cycle regulation of the cyclin A gene promoter is mediated by a variant E2F site. Proc. Natl. Acad. Sci. U.S.A. 92, 11264–11268.

Sontag, E., Fedorov, S., Kamibayashi, C., Robbins, D., Cobb, M., Mumby, M., 1993. The interaction of SV40 small t antigen with protein phosphatase 2A stimulates the Map kinase pathway and induces cell proliferation. Cell 75, 887–897.

Stubdal, H., Zalvide, J., DeCaprio, J.A., 1996. Simian virus 40 large T antigen alters the phosphorylation state of the RB-related proteins p130 and p107. J. Virol. 70, 2781–2788.

Stubdal, H., Zalvide, J., Campbell, K.S., Schweitzer, C., Roberts, T.M., DeCaprio, J.A., 1997. Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen. Mol. Cell. Biol. 17, 4979–4990.

Watanabe, G., Howe, A., Lee, R.J., Albanese, C., Shu, I.W., Karnezis, A.N., Zon, L., Kyriakis, J., Rundell, K., Pestell, R.G., 1996. Induction of cyclin D1 by simian virus 40 small tumor antigen. Proc. Natl. Acad. Sci. U.S.A. 93, 12861–12866.

Yang, S.-I., Lickteig, R.L., Estes, R., Rundell, K., Walter, G., Mumby, M.C., 1991. Control of protein phosphatase 2A by simian virus 40 small t antigen. Mol. Cell. Biol. 11, 1988–1995.

Yu, J., Boyapati, A., Rundell, K., 2001. Critical role for SV40 small-t antigen in human cell transformation. Virology 290, 192–198.

Yuan, H., Veldman, T., Rundell, K., Schlegel, R., 2002. Simian virus 40 small tumor antigen activates AKT and telomerase and induces anchorage-independent growth of human epithelial cells. J. Virol. 76, 10685–10691.

Zerfass-Thome, K., Schulze, A., Zwerschke, W., Vogt, B., Helin, K., Bartek, J., Henglein, B., Jansen-Durr, P., 1997. p27KIP1 blocks cyclin E-dependent transactivation of cyclin A gene expression. Mol. Cell. Biol. 17, 407–415.

Zerrahn, J., Knippschild, U., Winkler, T., Deppert, W., 1993. Independent expression of the transforming amino-terminal domain of large T antigen from an alternatively spliced third SV40 early mRNA. EMBO J. 12, 4739–4746.